



NASA ASTROBIOLOGY INSTITUTE

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Project Report: Early Metabolic Pathways

Lead Team:	<i>Ames Research Center</i>
Project Title:	<i>Early Metabolic Pathways</i>
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Project Progress

The origin of biological proteins is unclear; the complexity of even the simplest biological proteins has made their origin from random sequences seem difficult. We have developed a technology for in vitro protein evolution known as mRNA–display, and we have recently used this approach to select for functional proteins that bind adenosine triphosphate (ATP), starting from a very large library of random sequences. Characterization of these proteins indicates a high selectivity for ATP over GTP or cyclic ATP, while a functional requirement was observed for zinc ions, but not magnesium ions. Sequence analysis appears to implicate two conserved CXXC repeats that may be suggestive of a zinc finger protein folding motif. Biochemical characterization shows that these newly evolved proteins have very low folding energy, such that they are easily unfolded and easily become trapped in non–functional conformations. We have evolved variants of one of the ATP–binding proteins with more stable folded structures, using additional rounds of directed evolution under increasingly denaturing conditions. We are currently characterizing these proteins in a series of expression systems; our goal is to identify variants with improved solubility and thus suitability for structural studies. This will allow us to determine whether or not these de novo evolved proteins are structurally similar to biological proteins, and thus whether biological proteins use only a subset of possible protein folds.

In the previous period we had established a system that uses energy of sunlight to synthesize metabolically relevant compounds. It comprises liposomes containing oriented bacteriorhodopsin (for producing a proton gradient across the lipid membrane) and thermophilic ATPase (for producing ATP) upon illumination. The ATP produced could be utilized for synthesis of Acetyl–CoA. The next step was to encapsulate this proto–mitochondrion into a larger liposome representing a model protocell. Recently, we have been working on methods to encapsulate these bacteriorhodopsin liposomes into multi–lamellar vesicles (MULVS) containing pyranine, needed to detect pH changes inside the protocell. We varied the lipid composition of the latter vesicles, along with a temperature at which the encapsulation was done, as the MULVS proved to be very leaky. By varying these parameters we wanted

to avoid massive defects in the larger liposomes. So far, we see only very small pyranine signals, both because of the very low encapsulation extent and because of leakiness.

Highlights

- We have used directed evolution to evolve protein variants with increased stability of the functional folded state.
- Plans for next year include structure determination of newly evolved ATP-binding proteins of non-genomic origin for comparative studies with natural proteins of similar function.

Roadmap Objectives

- [**Objective No. 2: Origin of Life's Cellular Components**](#)
- [**Objective No. 3: Models for Life**](#)